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Abstract

The double-stranded RNA-binding domain (dsRBD) is a small protein domain found in eukaryotic, prokaryotic and viral proteins, whose central property is to bind to double-stranded RNA (dsRNA). Aside from this major function, recent examples of dsRBDs involved in the regulation of the sub-cellular localization of proteins, suggest that the participation of dsRBDs in nucleocytoplasmic trafficking is likely to represent a widespread auxiliary function of this type of RNA-binding domain. Overall, dsRBDs from proteins involved in many different biological processes have been reported to be implicated in nuclear import and export, as well as cytoplasmic, nuclear and nucleolar retention. Interestingly, the function of dsRBDs in nucleocytoplasmic trafficking is often regulated by their dsRNA-binding capacity, which can either enhance or impair the transport from one compartment to another. Here, we present and discuss the emerging function of dsRBDs in nucleocytoplasmic transport.

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1. Introduction

RNA performs a multitude of essential functions within the cell, many of which are achieved via interaction with proteins. For instance, many prokaryotic, eukaryotic and viral proteins interact with double-stranded RNA (dsRNA). This type of protein-RNA interaction regulates diverse biological processes such as response to viral infection, gene silencing through RNA interference pathways, RNA processing, regulation of translation, mRNA editing, RNA export and mRNA localization.[1-4] Proteins interacting with dsRNA often sense the double-helix RNA structure through one or multiple double-stranded RNA-binding domains (dsRBDs). The dsRBD (also referred to as dsRBM for double-stranded RNA-binding motif) is a conserved protein domain of approximately 65-70 amino acids in length, which binds double-stranded or highly structured RNAs. It was first recognized as a conserved protein domain from similarities between *Drosophila* Staufen, human TAR-RNA binding protein (TRBP) and *Xenopus laevis* RNA-binding protein A (XlrbpA).[5] The central function of dsRBDs is to bind to dsRNA regions. This is primarily achieved by recognizing the RNA shape, such as for in-

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stance the shape of a regular A-form RNA helix or the shape of an RNA hairpin, even though some dsRBDs can bind to dsRNA in a sequence-specific manner (see refs 4, 6-8 and below). Apart from this major function, recent examples of dsRBDs, often with protein-protein interaction properties, have been reported to participate in the regulation of the sub-cellular localization of proteins. This suggests that the participation of dsRBDs in nucleocytoplasmic trafficking is likely to represent a widespread auxiliary function of this type of RNA-binding domain. Among this increasing list of examples, we recently uncovered how the folding of one dsRBD of the human RNA-editing enzyme ADAR1 is assembling a bimodular nuclear localization signal responsible for the active transport of the protein towards the nucleus.[9] In this mini-review, after a brief description of RNA recognition by dsRBDs, we present and discuss the emerging function of dsRBDs in nucleocytoplasmic trafficking in the light of several examples that were recently disclosed.

2. Primary function of dsRBDs: dsRNA recognition

Although we will briefly highlight essential features of dsRNA recognition by dsRBDs, interested readers are referred to more comprehensive reviews on these aspects.[1,4,10] dsRBDs can be found in all kingdoms of life and are the second most abundant RNA binding domain,[1] after the RNA recognition motif (RRM), which is a well-characterized single-stranded RNA binding domain.[11] Proteins harboring the dsRBD vary considerably in function, depending on their catalytic domains or interaction partners. So far, the structures of more than 30 dsRBDs have been reported with a conserved α1-L1-β1-L2-β2-L3-β3-L4-α2 topology, where L specifies a loop (Figure 1A).[4,12] The second dsRBD of XlrbpA forms the prototype of canonical dsRBDs with the two α-helices packed against the three-stranded anti-parallel β-sheet.[13,14] In addition, structures of dsRBDs in complex with dsRNA substrates revealed the canonical mode of RNA recognition by dsRBDs.[6,15-17] Molecular recognition is accomplished via three distinct regions of interaction: helix α1 and the loop between β1 and β2 (L2) contact dsRNA minor grooves at one turn of interval, whereas the N-terminal tip of helix α2 contacts the dsRNA phosphate backbone across the major groove. Substrate recognition is achieved by virtue of solvent exposed residues in helix α1, the GPxH motif in the β1-β2 loop, and the positively charged KKxAK motif at the N-terminal tip of helix α2 (Figure 1A). Since the majority of dsRBD-RNA interactions involve direct contacts with the 2′-hydroxyl groups of the ribose sugar rings and direct- or water-mediated contacts with non-bridging oxygen of the phosphodiester backbone, the dsRBD is commonly described as a non-sequence specific RNA binding domain, which perfectly adapts to the shape of the A-form RNA helix.[1,2,18] In addition to this early description, it became clear that some dsRBDs display a pronounced preference for stem-loop structures, with the loop being often a primary determinant of RNA substrate recognition,[8,15,17,19] and that some dsRBDs perform a base-specific readout in the minor-groove via sequence specific contacts involving helix α1 and the β1-β2 loop.[4,7,20] One should therefore be aware that, beyond the A-form RNA helix, dsRBDs are sensitive to additional RNA features, such as apical loops, base-pair mismatches, bulges and nucleotide sequence.

3. dsRBDs and nuclear import

Some of the less conserved dsRBDs (also referred to as type-B dsRBDs)[21] have been shown to be involved in protein localization, while others are required for homo- and/or hetero-dimerization.[12,22,23] An interesting example of a type-B dsRBD involved in localization is the C-terminal dsRBD of human Dicer. Although processing of Dicer's substrates occurs in the cytoplasm of mammalian cells, several reports suggest that Dicer may also have additional roles in the nucleus.[24-27] In support of a nuclear function of Dicer, the C-terminal dsRBD of human Dicer was recently shown to harbor an atypical nuclear localization signal (NLS), composed of a cluster of positively charged residues on the surface of the folded dsRBD (Figure 1B).[28] The dsRBD-dependent import of Dicer follows different pathways and is mediated by Importin-β, Importin-7, and Importin-8.[28] However, in the full-length protein, the dsRBD-NLS is masked and Dicer localizes primarily to the cytoplasm. Therefore, even though the dsRBD-NLS mediates nuclear import, human Dicer constantly shuttles between the cytoplasm and the nucleus, and is primarily localized to the cytoplasm under steady-state conditions.[28] The shuttling properties of the dsRBD-NLS might be regulated by another domain of the protein situated in its N-terminal part, namely the helicase domain, and also by the presence of dsRNA.[28] It will there-

fore be interesting to investigate whether the localization of human Dicer is sensitive to variation of external conditions, a layer of regulation that was shown to enable a rapid response to stress conditions in the fission yeast.[29] We recently uncovered the molecular basis for the dsRBD-mediated nuclear import of the RNA-editing enzyme ADAR1.[9] Mammalian ADAR1 is a nucleocytoplasmic shuttling protein containing three dsRBDs. Nuclear import of ADAR1 is mediated by Transportin-1 (Trn1), a member of the karyopherin-β family, *via* an atypical NLS that overlaps the third dsRBD of the protein. This atypical NLS shows no similarity to known Trn1 NLSs, commonly referred to as PY-NLS.[30-33] The solution structure of the ADAR1-dsRBD3 revealed an extended dsRBD fold with an additional α-helix at its N-terminal part (Figure 1C).[9] This N-terminal extension radically changes the relative position of the flexible N- and C-terminal fragments flanking the dsRBD: it brings the N- and C-terminal flanking regions in close proximity (Figure 1C). The two flexible fragments flanking the folded dsRBD constitute two essential modules involved in the interaction with the import receptor Trn1 and thus the nuclear import of ADAR1. The two flanking modules form a so-called bimodular NLS where the two active modules are interrupted by a small folded domain. The intervening dsRBD was shown to only act as a scaffolding domain that properly positions the flanking modules for an effective interaction with Trn1 (Figure 1D). Indeed, functional bimodular NLSs could be designed by replacing the ADAR1-dsRBD3 with another unrelated dsRBD, or even with a small peptide linker that maintained the relative positioning of the N-and C-terminal modules. This clearly indicates that the dsRBD itself is not required for Trn1 interaction, but only helps juxtapose the two NLS-modules that are distantly spaced in the primary structure.[9] In addition, this impressive molecular construction was shown to act as an RNA-sensing NLS that can be switched *on* and *off*, depending on the presence of dsRNA associated with the dsRBD.[9] Indeed, the modulation of the import activity of ADAR1 directly depends on the RNA-binding status of the dsRBD-NLS.[9,33] The dsRBD-NLS presents two functional and non-overlapping interaction surfaces: (*i*) a functional dsRNA-binding interface that involves the canonical elements of dsRBDs for dsRNA binding; and (*ii*) a functional Trn1-binding interface that consists of the N- and C-terminal modules flanking the folded dsRBD (Figure 1E). Even though these two interfaces are distinct, the dsRBD-NLS cannot bind to dsRNA and Trn1 simultaneously, most probably for steric reasons, and therefore constitutes an RNA-sensing NLS.[9] Conversely, RNA-binding enhances nuclear export of ADAR1,[33] which overall positions RNA as a central regulator of ADAR1 shuttling. The fact that dsRNA stimulates nuclear export suggests that ADAR1 might leave the nucleus bound to substrate RNAs, such as precursors of micro-RNAs. The binding of the dsRBD-NLS to Trn1 in the cytoplasm could then help the dissociation of ADAR1-bound RNAs and ensure the reimport of ADAR1 free of RNAs to the nucleus, thereby preventing futile export cycles. In other words, binding of RNA to the RNA-sensing NLS of ADAR1 acts as a control of the directionality of transport, and prevents ADAR1 from carrying RNAs back into the nucleus.

4. dsRBDs and nuclear export

In eukaryotes, the segregation of nuclear and cytoplasmic compartments necessitates the continuous transport of large macromolecules across the nuclear envelop for the proper cellular function.[34,35] Many post-transcriptional functions of RNAs occur in the cytoplasm, far from their nuclear site of biogenesis. Importantly, dsRBDs have been reported to be involved in the export of proteins and/or RNAs through direct or indirect interactions with export factors such as Exportin-5 (Exp5) (see references below). Exp5 is a member of the human karyopherin-β family, which shuttles cargo macromolecules from the nucleus to the cytoplasm in a Ran-dependent manner. dsRBD-dependent nuclear export by Exp5 has been reported for many dsRBD-containing proteins such as the elongation factor 1A (eEF1A), the interleukin enhancer binding factor-3 (ILF3), the nuclear factor 90 (NF90), Staufen proteins, and the short isoform of the human editing enzyme ADAR1.[33,36-40] Whether dsRBDs bind to Exp5 directly or *via* a dsRNA bridge seems to vary from one protein to another, and both situations have been reported in the literature (see the three examples below).[33,36-38] For instance, in human ILF3, the second dsRBD of the protein was first proposed to mediate nuclear export through a direct interaction with Exp5,[38] but this interaction, and therefore the nuclear export of the protein, was latter shown to be mediated by the binding of dsRBDs to dsRNA.[36,37] Staufen proteins represent another interesting example of a dsRBD involved in nuclear

export. Staufen localizes mRNAs in *Drosophila*, and is conserved from worms to humans. Staufen proteins possess 4 to 5 dsRBDs depending on the species and protein isoform. It has been reported that nuclear export of mammalian Staufen2 occurs *via* the interaction of the third dsRBD of the protein with Exp5.[39,40] This interaction is abolished in a mutant deficient in RNA-binding, which supports the model of a dsRNA bridge for this dsRBD to interact with Exp5.[39,40] Conversely, the Exp5-mediated nuclear export of the shorter isoform of ADAR1 depends on the interaction of Exp5 with the first dsRBD of the protein, an interaction that is enhanced in the presence of RNA, but persists upon RNase treatment.[33] Therefore, although dsRNA stimulates nuclear export of ADAR1, it seems not essential and binding of dsRBDs to Exp5 seemingly occurs directly.[33] In any case, although the implication of dsRBDs in nuclear export of proteins is often mediated by their RNA-binding properties, their function in nuclear export is central for the subcellular localization of many dsRBD-containing proteins.

5. dsRBDs in nuclear or cytoplasmic retention

Another mechanism influencing the subcellular localization of proteins is through modulation of the binding of these proteins to specific nuclear or cytoplasmic factors that anchor, or in other words retain the protein of interest within a specific cytoplasmic or nuclear compartment. The elements involved in the interaction with the anchoring factors are called retention signals, and in the case of dsRBD-containing proteins, dsRBDs have been shown to participate in both nuclear and cytoplasmic retention (see below and references therein). Indeed, similarly to human Dicer, for which the C-terminal dsRBD was shown to be involved in the regulation of the sub-cellular localization of the protein, and especially its transient nuclear import (see ref 28, and text above), the C-terminal dsRBD of the yeast *Schizosaccharomyces pombe* Dicer (Dcr1) is also critical for the nuclear localization of the protein.[41] In contrast to human Dicer, *S. pombe* Dcr1 is predominantly nuclear and localizes at the nuclear periphery, near the nuclear pore complexes (NPCs).[41] Similarly to human Dicer, Dcr1 has the potential to shuttle between the nucleus and cytoplasm, and its C-terminal dsRBD orchestrates its nuclear retention.[41,42] Interestingly, Dcr1's dsRBD folds as an extended dsRBD with an essential C-terminal extension composed of a short α-helical turn followed by a zinc-coordination motif of the CHCC type (Figure 1F).[42] Strikingly, only the last two ligands (chCC) are found within the extension, whereas the first two ligands (CHcc) are part of the dsRBD fold itself (respectively in loops L1 and L3). With this particular structure, the extended dsRBD presents a protein-protein interaction surface (Figure 1G), which mediates nuclear retention of Dcr1, by interacting with an unidentified nuclear protein that probably drives its localization at the nuclear periphery.[41,42] Importantly, the extended dsRBD of Dcr1 is a temperature sensitive domain, which acts as a thermoswitch, where elevated temperature above 34-38 °C result in an alteration of the nuclear retention properties of the dsRBD. As a result, Dcr1 localizes in the cytoplasm at elevated temperature, with important consequences for silencing and RNAi-mediated response to stress conditions.[29] Additionally, cytoplasmic retention mediated by dsRBDs has also been reported in a few proteins. For instance, in mammalian Staufen1, dsRBD3 plays critical roles in the cytoplasmic retention of the protein.[43] This retention activity is enhanced in the presence of dsRBD2, and noteworthy, independent of their RNA-binding capacity.[43] Conversely, cytoplasmic retention of the *Xenopus* transcription factor CBTF was also shown to require both its' dsRBDs, but is directly driven by the dsRNA-binding capacity of these domains.[44] Finally, within the nucleus, several dsRBDs have been shown to guide proteins to the nucleolus and to mediate their retention in this compartment. This property was reported for many distinct proteins, such as XlrbpA,[45] ADAR proteins,[46-48] and PKR.[49] The targeting of these proteins to the nucleolus is likely driven by their ability to bind to ribosomal RNA.[45-49] For most proteins, it is largely unknown whether the nucleolar localization only reflects their dsRNA-binding capacity, or whether it has a direct biological function.

6. Conclusion and perspectives

In this mini-review, we underlined the fact that many dsRBDs play a critical role in the subcellular localization of the protein they belong to. This small and compact domain has been reported to mediate nuclear import and export, as well as cytoplasmic, nuclear, and even nucleolar retention (see Figure 2 for a schematic representation of the diverse functions of dsRBDs in nucleocytoplasmic transport

and for a list of the currently known examples of proteins for which dsRBDs are involved in their sub-cellular localization). Attractively, in the few cases where involvements of dsRBDs in nucleocytoplasmic transport were investigated structurally, extensions to the dsRBD fold have often been described, and these N- or C-terminal extensions were shown to either directly or indirectly participate in the regulation of the protein nucleocytoplasmic distribution.[9,42] In addition, these extended dsRBDs fully retain the capacity to bind to dsRNA, suggesting that extending the dsRBD fold would be a widespread strategy to acquire additional functions without losing the dsRNA-binding activity. More generally, the function of dsRBDs in trafficking is often regulated by their dsRNA-binding capacity, which can either enhance or impair the transport from one compartment to another. For instance, in the case of ADAR1, the competition between the import receptor Trn1 and dsRNA allows the nuclear import to be switched *on* and *off* depending on the presence of RNA associated with the dsRBD-NLS.[9] In our opinion, combining folded functional domains and localization signals is a very attractive molecular construction enabling a myriad of possibilities for the subtle regulation of cellular localization. In a near future, we believe that the list of proteins with a dsRBD involved in subcellular transport and localization will keep growing, and that new aspects of the function of dsRBDs in nucleocytoplasmic transport will surely be uncovered.

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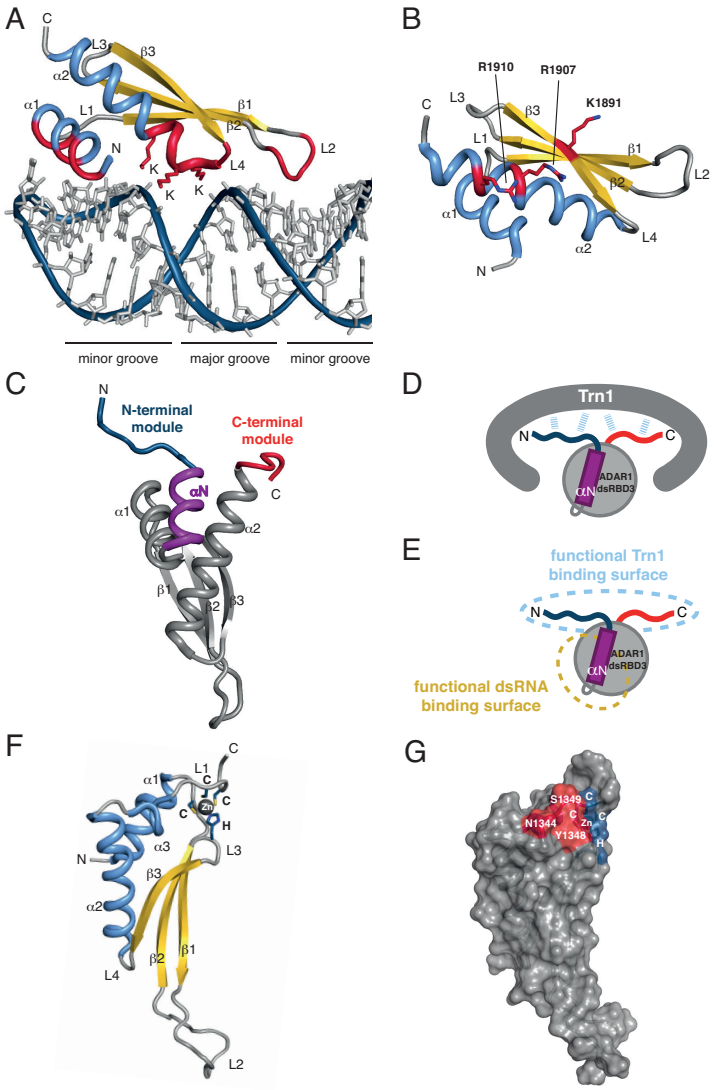

Figure 1. Structural aspects of the functions of double-stranded RNA-binding domains.
(**A**) Primary function of dsRBDs as dsRNA binders. Cartoon representation of the canonical mode of interaction of dsRBDs with dsRNA (ADAR2-dsRBD1 is shown as an example; PDB code 2l3c).[7] β-strands are in yellow and α-helices in blue. The three regions of interaction with dsRNA are in red, with the three conserved lysines of the so-called KKxAK motif shown as sticks. Helix α1 and the loop L2 interact with dsRNA minor grooves at one turn of interval. The N-terminal tip of helix α2 interacts with the intervening major groove. (**B**) Cartoon representation of the C-terminal dsRBD of human Dicer (PDB code 3c4b). The atypical dsRBD-NLS of human Dicer is composed of a cluster of positively charged residues represented as sticks in red (namely K1891, R1910 and R1907).[28] Noteworthy, these residues do not belong to the canonical region of interaction with dsRNA. (**C**) Cartoon representation of the dsRBD-NLS of human ADAR1 (PDB code 2mdr).[9] The atypical dsRBD-NLS of human ADAR1 is composed of the N- and C-terminal modules (in blue and in red, respectively) flanking the folded dsRBD. The additional N-terminal helix (helix αN, in purple) brings the two modules in close proximity. (**D**) Schematic representation of the interaction of the ADAR1 dsRBD-NLS with Trn1. The dsRBD core domain is in gray, the N- and C-terminal modules are in blue and in red, respectively, and the additional helix αN is in purple. Transportin-1 is represented as a gray arch. (**E**) Schematic representation of the two functional binding interfaces of the ADAR1 dsRBD-NLS. These two binding sites cannot be occupied simultaneously, most probably for steric reasons, and therefore, the dsRBD-NLS constitutes an RNA-sensing NLS.[9] (**F**) Cartoon representation of the C-terminal dsRBD of *S. pombe* Dicer (Dcr1, PDB code 2l6m).[42] β-strands are in yellow and α-helices in blue. Dcr1's dsRBD folds as an extended dsRBD with a C-terminal extension composed of a short α-helical turn (helix α3) and a zinc-coordination motif of the CHCC type. The zinc ion is shown as a gray sphere, and the CHCC ligands as blue sticks. (**G**) The C-terminal extension of Dcr1's dsRBD provides an additional surface, and the residues highlighted in red (namely N1344, Y1348 and S1349) form a protein-protein interaction surface that is required to retain Dcr1 in the nucleus.[42]

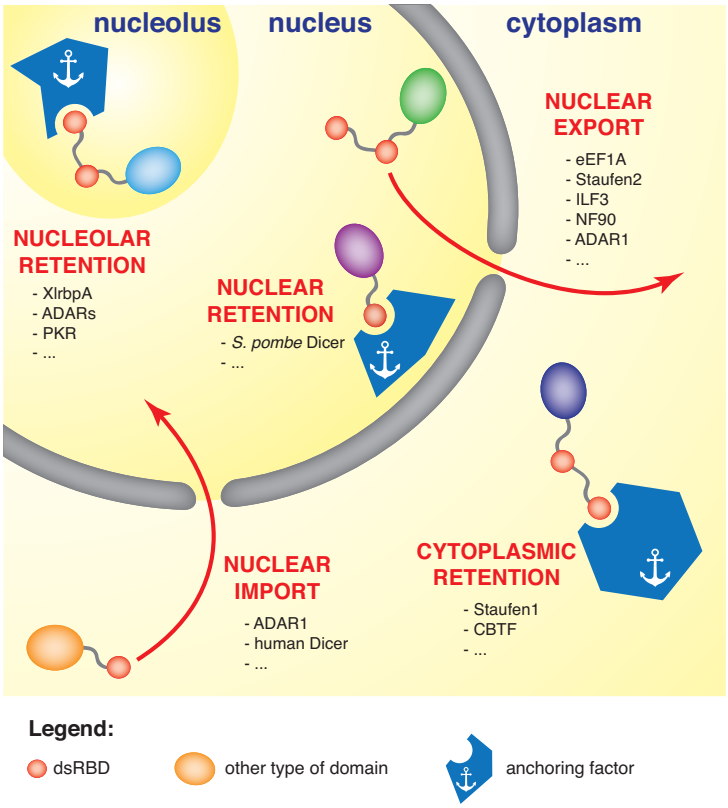

Figure 2. Functions of double-stranded RNA-binding domains in nucleocytoplasmic transport. dsRBDs (red spheres) are involved in many aspects governing the subcellular localization of proteins. This includes nuclear import and export (symbolized with red arrows), as well as cytoplasmic, nuclear, and nucleolar retention *via* the interaction with anchoring factors (blue polygons). Other types of protein domains are symbolized as spheres of various colors. For each function of dsRBDs in nucleocytoplasmic transport (text in red), the protein examples such as presented in this mini-review are listed below (text in black). This list does not aim at being exhaustive and is likely to increase in the near future.